

AMENDMENTS TO THE CLAIMS

51-60. Withdrawn.

61. (Currently Amended) A method of producing a targeting construct, the method comprising:

- (a) providing a polynucleotide homologous to a target sequence;
- (b) generating two different fragments of the polynucleotide, wherein each of the fragments ~~having~~ have single-stranded ends which are complementary to a vector having a gene encoding a positive selection marker;
- (c) providing the vector having a gene encoding a positive selection marker; and
- (d) using ligation independent cloning to insert the two different fragments into the vector to form the construct, wherein the positive selection marker is positioned between the two different fragments in the construct.

62. (Previously Added) The method of claim 61, wherein the positive selection marker is a neomycin resistance gene.

63. (Previously Added) The method of claim 61, wherein the vector comprises the sequence set forth in SEQ ID NO:1 or the sequence set forth in SEQ ID NO:2.

64. (Previously Added) The method of claim 61, wherein the vector comprises a sequence encoding a screening marker.

65. (Previously Added) The method of claim 64, wherein the screening marker is a fluorescent protein.

66. (Previously Added) The method of claim 61, wherein the vector further comprises a sequence encoding a negative selection marker.

67. (Previously Added) The method of claim 66, wherein the negative selection marker is thymidine kinase.

68. (Currently Amended) The method of claim 61, wherein the polynucleotide sequence of step (a) is obtained by PCR amplifying the two different fragments of step (b) with oligonucleotide primers having 5' sequences lacking one type of base and are at least 12 nucleotides in length.

69. (Currently Amended) The method of claim 68, wherein the oligonucleotide primers ~~are comprised of~~ comprise the sequences set forth in SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5, SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; or SEQ ID NO:10.

70. (Previously Added) The method of claim 61, wherein the ligation independent cloning is performed in one step.

71. (Previously Added) The method of claim 61, wherein the ligation independent cloning is performed in more than one step.

72. (Previously Added) The method of claim 61, wherein the polynucleotide is isolated from a plasmid library.

73. (Previously Added) A method of producing a targeting construct, the method comprising:

- (a) providing a circular plasmid library;
- (b) isolating a polynucleotide sequence from the library using oligonucleotide primers having 5' sequences lacking one type of base, the polynucleotide sequence comprising a first region and a second region of a target sequence;
- (c) generating a first fragment comprising the first region and a second fragment comprising the second region;

- (d) providing a vector having a gene encoding a positive selection marker; and
- (e) inserting the first fragment and second fragment into the vector to form the construct, wherein the positive selection marker is positioned between the first fragment and second fragment in the construct.

74. (Previously Added) The method of claim 73, wherein the first and second fragments are inserted using ligation-independent cloning.

75. (Previously Added) The method of claim 73, wherein the positive selection maker is a neomycin resistance gene.

76. (Previously Added) The method of claim 73, wherein the vector comprises the sequence set forth in SEQ ID NO:1 or the sequence set forth in SEQ ID NO:2.

77. (Previously Added) The method of claim 73, wherein the vector comprises a sequence encoding a screening marker.

78. (Previously Added) The method of claim 73, wherein the screening marker is a fluorescent protein.

79. (Previously Added) The method of claim 73, wherein the vector further comprises a sequence encoding a negative selection marker.

80. (Previously Added) The method of claim 73, wherein the negative selection marker is thymidine kinase.

81. (Currently Amended) The method of claim 73, wherein the oligonucleotide primers ~~are comprised of~~ comprise the sequences set forth in SEQ ID NO:3; SEQ ID NO:4; SEQ ID NO:5; SEQ ID NO:6; SEQ ID NO:7; SEQ ID NO:8; SEQ ID NO:9; or SEQ ID NO:10.

82. (Previously Added) The method of claim 73, wherein the oligonucleotide primers are at least 12 nucleotides in length.
